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Lessons Learned from Inherited Metabolic Disorders of Sulfur-Containing Amino Acids Metabolism

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ABSTRACT

The metabolism of sulfur-containing amino acids (SAAs) requires an orchestrated interplay among several dozen enzymes and transporters, and an adequate dietary intake of methionine (Met), cysteine (Cys), and B vitamins. Known human genetic disorders are due to defects in Met demethylation, homocysteine (Hcy) remethylation, or cobalamin and folate metabolism, in Hcy transsulfuration, and Cys and hydrogen sulfide (H_2S) catabolism. These disorders may manifest between the newborn period and late adulthood by a combination of neuropsychiatric abnormalities, thromboembolism, megaloblastic anemia, hepatopathy, myopathy, and bone and connective tissue abnormalities. Biochemical features include metabolite deficiencies (e.g. Met, *S*-adenosylmethionine (AdoMet), intermediates in 1-carbon metabolism, Cys, or glutathione) and/or their accumulation (e.g. *S*-adenosylhomocysteine, Hcy, H_2S, or sulfite). Treatment should be started as early as possible and may include a low-protein/low-Met diet with Cys-enriched amino acid supplements, pharmacological doses of B vitamins, betaine to stimulate Hcy remethylation, the provision of *N*-acetylcysteine or AdoMet, or experimental approaches such as liver transplantation or enzyme replacement therapy. In several disorders, patients are exposed to long-term markedly elevated Met concentrations. Although these conditions may inform on Met toxicity, interpretation is difficult due to the presence of additional metabolic changes. Two disorders seem to exhibit Met-associated toxicity in the brain. An increased risk of demyelination in patients with Met adenosyltransferase I/III (MATI/III) deficiency due to biallelic mutations in the *MATIA* gene has been attributed to very high blood Met concentrations (typically >800 μmol/L) and possibly also to decreased liver AdoMet synthesis. An excessively high Met concentration in some patients with cystathionine β-synthase deficiency has been associated with encephalopathy and brain edema, and direct toxicity of Met has been postulated. In summary, studies in patients with various disorders of SAA metabolism showed complex metabolic changes with distant cellular consequences, most of which are not attributable to direct Met toxicity. *J Nutr* 2020;150:2506S–2517S.

Keywords: homocystinuria, cystathionine β-synthase deficiency, methionine adenosyltransferase I/III deficiency, glycine *N*-methyltransferase deficiency, *S*-adenosylhomocysteine hydrolase deficiency, adenosine kinase deficiency, remethylation defects, ethylmalonic encephalopathy, sulfite oxidase deficiency, methionine restricted diet

Introduction

Role of methionine and cysteine in metabolism

Methionine (Met) is an essential sulfur-containing amino acid (SAA). This low abundance proteinogenic amino acid is involved in translational initiation, sequence-independent recognition of protein surfaces, and reversible redox regulation of protein function via methionine sulfoxide formation (1). In addition, Met is needed for the synthesis of *S*-adenosylmethionine (AdoMet) (2), a donor of methyl groups for >300 human methyltransferases (3), and a precursor for polyamine synthesis. Cysteine (Cys) is a semiessential SAA which plays an important role in protein structure via the formation of disulfide bridges and metal-binding sites, in catalysis of nucleophilic and redox reactions, and allosteric regulation (4, 5). Cys is also a precursor for the synthesis of physiologically active compounds such as hydrogen sulfide (H_2S), glutathione (GSH), and taurine. For details on biochemical pathways and their regulation see a recent review (6).

Human genetic disorders in metabolism of SAA

Functional metabolism of SAAs requires an adequate intake of Met and/or Cys, sufficient supply of B vitamins, and an orchestrated transcriptional and allosteric regulation of several dozen enzymes and transporters (7). Pathophysiology and clinical aspects of disorders manifesting by hypermethioninemia has been published elsewhere (8). At present, 4 groups of

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monogenic disorders of SAA metabolism have been described in humans (for overview see **Figure 1**, **Table 1**, and **Table 2**), namely defects in Met transmethylation, disorders of homocysteine (Hcy) remethylation including defects in cobalamin and folate processing and transport, diseases in the transsulfuration pathway of Hcy, and disorders of Cys and H_2S metabolism. The population frequency of these autosomal recessive (AR) disorders is largely unknown, but estimates for individual diseases range between 1:1800 for homocystinuria in Qatar to 1 in several million for the ultrarare conditions (9, 10). This short review summarizes key biochemical and clinical aspects of known human genetic disorders affecting SAA metabolism.

Disorders in the Transmethylation Pathway

General characteristics

The transmethylation pathway of Met is vitally important for the production of AdoMet, which yields Hcy as an end product. Inherited enzyme deficiencies in transmethylation are generally characterized by moderate to severe hypermethioninemia with varying changes in AdoMet, *S*-adenosylhomocysteine (AdoHcy), adenosine, Hcy, and *N*-methylglycine metabolism (11, 12). The main mechanisms implied in this group of disorders [except in methionine adenosyltransferase I/III (MATI/III) deficiency] relate to toxic effects of Met, disturbed signaling cascades, and altered methylation reactions with predominant brain and liver involvement (12–14).

MATI/III deficiency

The MATI/III enzyme is present in hepatocytes and contributes to the maintenance of whole-body AdoMet homeostasis (8, 15). Both genetically distinct forms of MATI/III deficiency lead to severe hypermethioninemia (8, 16, 17). The autosomal dominant type of MATI/III deficiency is caused by specific mutations with a dominant negative effect on the oligomeric structure of the enzyme; individuals with this form of MATI/III deficiency are usually asymptomatic and most of them were detected unintentionally by newborn screening for classical homocystinuria due to markedly elevated Met concentrations in dried blood spots (17, 18–20). The AR form—caused by biallelic mutations in the *MAT1A* gene—exhibits, in addition to severe hypermethioninemia, slightly subnormal to normal AdoMet and slightly elevated Hcy in blood. Neurological sequelae have been observed in about half of patients, and a typical finding is brain demyelination with edema of subcortical and deep white matter resulting in separation of myelin layers, i.e. vacuolating myelinopathy (16, 21). The previous analysis of 64 cases indicates that neurological and subclinical MRI abnormalities occur predominantly in patients with plasma Met concentrations above 800 μmol/L, whereas these complications are rare in subjects with lower Met concentrations (17). No laboratory or clinical signs of hepatopathy have been observed in this cohort. Treatment has been recommended only in symptomatic patients with the AR form or in asymptomatic individuals with plasma Met >500–800 μmol/L. Dietary Met restriction and the avoidance of betaine and possibly AdoMet supplementation are the recommended therapeutic measures (11).

Methionine adenosyltransferase II deficiency

Methionine adenosyltransferase II (MATII) is a ubiquitously expressed enzyme providing AdoMet for methylation reactions in tissues. No patients with biallelic mutations in the *MAT2A* gene have been described so far and only heterozygous mutations have been detected as a low penetrance risk factor for developing thoracic aortic aneurysm (22). The mechanism behind this association is elusive and the effect on Met-related metabolism has not been reported.

Methanethiol oxidase deficiency

Met can also be transaminated to yield methanethiol (also produced by the intestinal microbiota), which is further converted to H_2S by methanethiol oxidase (MTO). A deficiency of MTO leads to the accumulation of malodorous molecules such as methanethiol and dimethysulfide and extraoral halitosis, this condition has been described in only 5 patients (23).

Glycine *N*-methyltransferase deficiency

Glycine *N*-methyltransferase (GNMT) is expressed in the liver and several other organs and maintains AdoMet homeostasis by converting surplus AdoMet to *N*-methylglycine (24). GNMT deficiency is an ultrarare disease that has been described in only 5 young patients (13, 25) with markedly elevated blood Met and AdoMet, and normal *N*-methylglycine and AdoHcy concentrations. Although GNMT-deficient mice develop liver disease and hepatocellular carcinoma (26, 27), human patients exhibit only a mild to moderate hepatopathy with elevated aminotransferases and mild hepatomegaly. There is no evidence that therapy using a low-Met diet is beneficial, however, it may be considered in patients with plasma Met >500–600 μmol/L (11).

Adenosine kinase deficiency

Adenosine kinase (ADK) converts adenosine to AMP. ADK deficiency leads to the accumulation of adenosine and hypoxanthine and to moderately to markedly elevated blood

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Abbreviations used: ADK, adenosine kinase; AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; AR, autosomal recessive; CBS, cystathionine β-synthase; Cys, cysteine; E-HOD, European Registry for Homocystinurias and Methylation Defects; GNMT, glycine *N*-methyltransferase; GSH, glutathione; H_2S, hydrogen sulfide; Hcy, homocysteine; MATI/III, methionine adenosyltransferase I/III; Met, methionine; MTO, methanethiol oxidase; MTR, methionine synthase; MTRR, methionine synthase reductase; Na^+/K^+ ATPase, sodium/potassium ATPase; PLP, pyridoxal 5′-phosphate; SAA, sulfur-containing amino acid; SUOX, sulfite oxidase.

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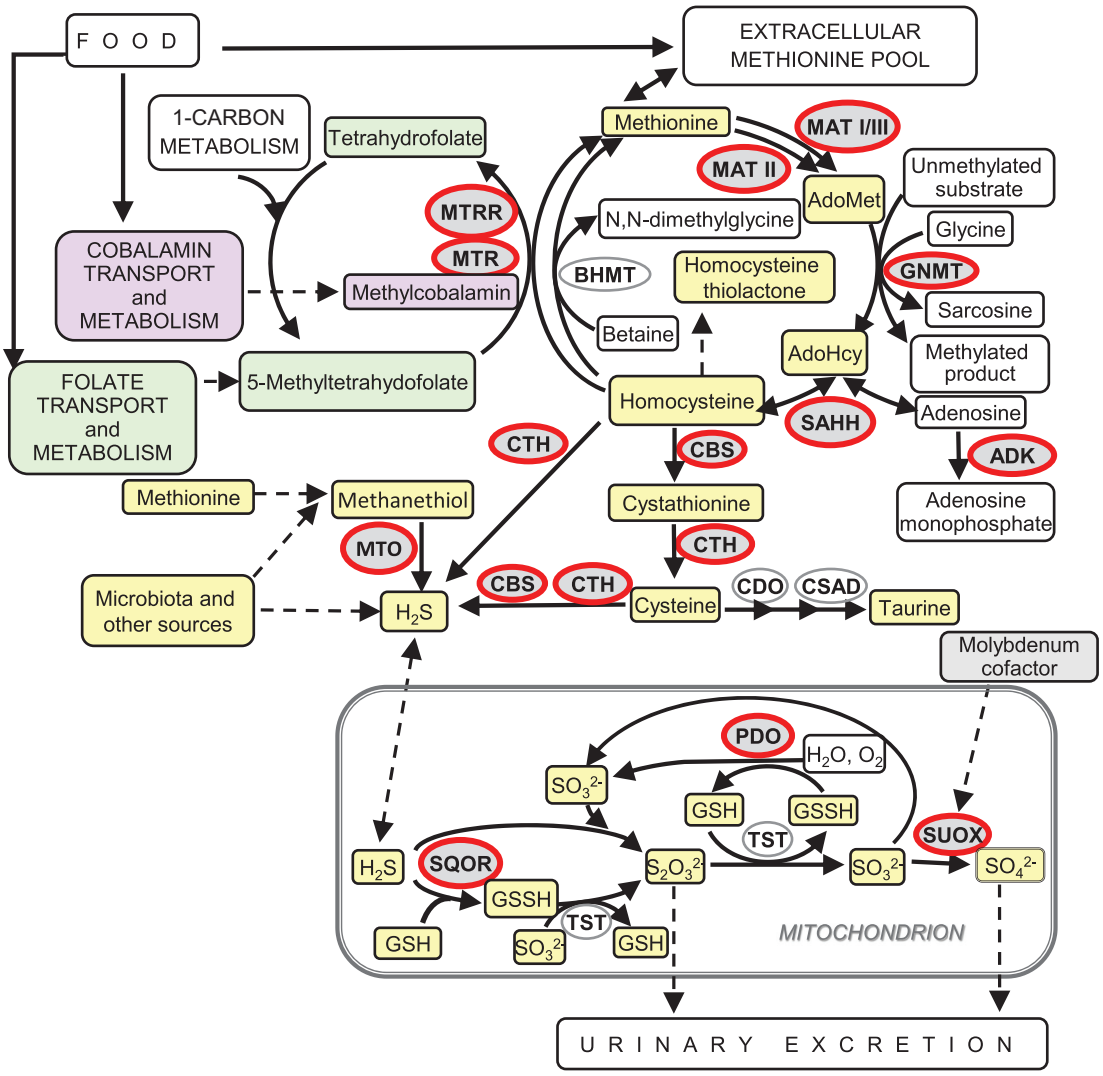

FIGURE 1 Metabolism of sulfur-containing amino acids and related compounds (enzymes deficient in disorders discussed in this article are marked in red). Methionine is converted to homocysteine by reactions involving methionine adenosyltransferase I/III, methionine adenosyltransferase II, and *S*-adenosylhomocysteine hydrolase. Excess of AdoMet is converted to sarcosine by glycine *N*-methyltransferase. Homocysteine can be remethylated back to methionine by betaine: homocysteine methyltransferase or by a complex of methionine synthase and methionine synthase reductase, which requires methyl groups from folate and cobalamin metabolism. Homocysteine can be converted to cysteine by cystathionine β-synthase and cystathionine γ-lyase. Cysteine serves as a precursor for synthesis of hydrogen sulfide catalyzed by CBS, CTH, aspartate aminotransferase, and mercaptopyruvate sulfurtransferase (not shown); hydrogen sulfide may also be synthesized by methanethiol oxidase. Oxidation of cysteine to taurine is catalyzed by cysteine dioxygenase and cysteine sulfinic acid decarboxylase. Mitochondrial oxidation of hydrogen sulfide to sulfite and finally to sulfate starts by synthesis of the intermediate glutathione persulfide catalyzed by sulfide: quinone oxidoreductase followed by interconversions catalyzed by persulfide dioxygenase, thiosulfate transferase and sulfite oxidase. ADK, adenosine kinase; AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; BHMT, betaine: homocysteine methyltransferase; CBS, cystathionine β-synthase; CDO, cysteine dioxygenase; CSAD, cysteine sulfinic acid decarboxylase; CTH, cystathionine γ-lyase; GNMT, glycine *N*-methyltransferase; GSSH, glutathione persulfide; H_2S, hydrogen sulfide; MATII, methionine adenosyltransferase II; MATI/III, methionine adenosyltransferase I/III; MTO, methanethiol oxidase; MTR, methionine synthase; MTRR, methionine synthase reductase; PDO, persulfide dioxygenase; SAHH, *S*-adenosylhomocysteine hydrolase; SO_3^{2-}, sulfite; SO_4^{2-}, sulfate; $S_2O_3^{2-}$, thiosulfate; SQOR, sulfide: quinone oxidoreductase; SUOX, sulfite oxidase; TST, thiosulfate transferase.

AdoHcy, Hcy, and Met concentrations. In a cohort of 19 patients, ADK deficiency manifested by bone abnormalities (macrocephaly, frontal bossing, hypertelorism, and cardiac anomalies), neurological complications (severe developmental delay, hypotonia, and epilepsy), failure to thrive, and liver disease (neonatal jaundice, elevated aminotransferases, and impaired coagulation) (11, 28, 29). A low-Met diet ameliorates the liver phenotype but the beneficial effect on neurological outcome has been reported in only a single case. Patients may also be treated by diazoxide for recurrent hypoglycemia resulting from hyperinsulinism of unclear origin (11).

AdoHcy hydrolase deficiency

AdoHcy hydrolase catalyzes a bidirectional reaction, which under physiological conditions favors AdoHcy hydrolysis to Hcy and Ado. This ultrarare disease has been reported in 10 patients (30, 31–33). Biochemical hallmarks include markedly elevated AdoHcy and AdoMet, normal to moderately elevated Hcy,

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TABLE 1 Key biochemical abnormalities in disorders of sulfur amino acid metabolism

Enzyme deficiency/disorder	Gene	Met	AdoMet	Sarcosine (*N*-methylglycine)	AdoHcy	Total Hcy	Other metabolites	References
MATI/III deficiency, AD form	*MAT1A*	↑↑-↑↑↑	n	N	N	n		(8, 34)
MATI/III deficiency, AR form	*MAT1A*	↑↑↑	n-↓	N	N	n-↑	Methionine sulfoxide ↑	(8, 16, 17, 34, 35, 36)
Thoracic aneurysm	*MAT2*	N/A	N/A	N/A	N/A	N/A		(22, 34, 37)
MTO deficiency	*SELENBP1*	N/A	N/A	N/A	N/A	N/A	Methanthiol ↑, dimethylsulfide ↑, dimethylsulfoxide ↑	(34, 37)
GNMT deficiency	*GNMT*	↑↑-↑↑↑	↑↑-↑↑↑	n	n-↑	n-↑		(8, 15, 25, 34, 36, 37)
ADK deficiency	*ADK*	n-↑↑↑	↑-↑↑	N/A	↑-↑↑	n-↑	Adenosine n-↑	(29, 34, 36, 37)
SAHH deficiency	*AHCY*	n-↑↑↑	↑↑-↑↑↑	n-↑	↑↑-↑↑↑	n-↑		(8, 30, 34, 36, 37)
B-vitamin transport and remethylation defects	≈30 genes	n-↓	n-↓	n-↑	↑↑	↑↑	Methylmalonic acid n-↑↑↑ and Hcy thiolactone ↑↑↑	(38, 39, 34, 36, 40, 41)
CBS deficiency	*CBS*	n-↑↑↑	↑-↑↑	↑-↑↑	↑↑-↑↑↑	↑↑-↑↑↑	Cystathionine n-↓↓, total Cys↓, Hcy thiolactone ↑↑↑, H_2S n-↑	(8, 38, 42, 39, 34, 36, 37, 43)
CTH deficiency	*CTH*	n	N/A	n-↑	N/A	n-↑	Cystathionine ↑↑↑, total Cys n	(44, 34, 36, 37, 43)
SQOR deficiency	*SQOR*	N/A	N/A	N/A	N/A	N/A	Lactate ↑	(45, 34)
Ethylmalonic encephalopathy	*ETHE1*	n	N/A	n-↑	N/A	n-↓	H_2S ↑, sulfite ↑↑-↑↑↑, thiosulfate ↑↑-↑↑↑, *S*-sulfocysteine, and *S*-sulfohomocysteine ↑↑-↑↑↑, lactate and short chain acylcarnitines ↑-↑↑	(46, 34, 36, 37, 43)
SUOX deficiency and molybdenum cofactor deficiencies	*SUOX, MOCS1, MOCS2, GEPHN*	n	N/A	n-↑	N/A	↓-↓↓	H_2S n-↑, sulfite ↑↑↑, thiosulfate ↑↑↑, *S*-sulfocysteine and *S*-sulfohomocysteine ↑↑↑, uric acid ↓-↓↓and xanthine ↑-↑↑ (only in molybdenum cofactor deficiencies)	(47, 34, 36, 37, 43)
Typical reference ranges in plasma (across ages, μmol/L)		12–40	0.03–0.16	0.6–2.5	0.015–0.06	3.5–15		

Arrows indicate typical changes in concentrations of metabolites in body fluids, n, normal values, ↑-between upper limit and ≈ 5 × UL, ↑↑- ≈ 5×-1 ≈ 0 × UL, ↑↑↑- above 1 ≈ 0× UL, ↓ between 0 ≈ 0.5× lower limit and lower limit, ↓↓ below ≈ 0.5× lower limit. AD, autosomal dominant; ADK, adenosine kinase; AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; AR, autosomal recessive; CBS, cystathionine β-synthase; CTH, γ-cystathionase; Cys, cysteine; EMA, ethylmalonic aciduria; GNMT, glycine *N*-methyltransferase; H_2S, hydrogen sulfide; Hcy, homocysteine; MATI/III, methionine adenosyltransferase I/III; MATII, methionine adenosyltransferase II; Met, methionine; MTO, methanethiol oxidase; N/A, data not available; SAHH, *S*-adenosylhomocysteine hydrolase; SQOR, sulfide: quinone oxidoreductase; SUOX, sulfite oxidase; UL, upper limit.

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TABLE 2 Key clinical abnormalities observed in patients with disorders of sulfur amino acid metabolism

Enzyme deficiency/disorder	Liver and digestive system	Nervous system	Cardiovascular system and blood	Bones and connective tissue	Other systems
MATI/III deficiency, AD form	–	–	–	–	–
MATI/III deficiency, AR form	–	Brain demyelination, cognitive and movement disturbances	–	–	Cabbage-like breath odor
Thoracic aneurysm due to *MAT2A* heterozygosity	–	–	Thoracic aneurysm	–	–
MTO deficiency	–	Inconstant cognitive problems	–	–	Cabbage-like breath odor
GNMT deficiency	Hepatomegaly and elevated aminotransferases	–	–	–	–
ADK deficiency	Hepatopathy with steatosis and cholestasis, failure to thrive	Developmental delay, cognitive impairment, epilepsy	Cardiac anomalies	Macrocephaly, hypertelorism, frontal bossing, short stature	Hypoglycemia due to hyperinsulinism
SAHH deficiency	Hepatopathy, hepatocellular carcinoma	Developmental delay, hypoplasia of brain structures, behavioral changes	–	–	Myopathy with muscle weakness, severe cases manifest by fetal hydrops
B-vitamin transport and remethylation defects	No major complications to failure to thrive	Impaired myelination, cognitive defects, spasticity retinopathy	Thromboembolism, macrocytic/megaloblastic anemia, hemolytic uremic syndrome in some disorders	–	
CBS deficiency	Clinically inapparent mild hepatopathy	Cognitive impairment, behavioral problems, psychosis, seizures	Thromboembolism	Osteoporosis, marfanoid features, kyphoscoliosis, lens dislocation	–
CTH deficiency	–	Inconstant cognitive problems	–	–	Considered benign condition
SQOR deficiency	–	Episodic coma with brain MRI changes mimicking Leigh syndrome	–	–	Lethal multiorgan failure
Ethylmalonic encephalopathy	Chronic hemorrhagic diarrhea	Seizures, cognitive impairment, brain MRI changes mimicking Leigh syndrome	Orthostatic acrocyanosis, petechiae	–	Hematuria
SUOX deficiency and molybdenum cofactor deficiencies	Feeding difficulties	Severe brain atrophy with cysts, cognitive impairment, intractable seizures	–	Lens dislocation	Urolithiasis (only in molybdenum cofactor deficiency)

–, Clinical abnormalities for these organs were not reported. AD, autosomal dominant form; ADK, adenosine kinase; AR, autosomal recessive form; CBS, cystathionine β-synthase; CTH, γ-cystathionase; GNMT, glycine *N*-methyltransferase; MATI/III, methionine adenosyltransferase I/III; MTO, methanethiol oxidase; SAHH, *S*-adenosylhomocysteine hydrolase; SQOR, sulfide: quinone oxidoreductase; SUOX, sulfite oxidase.

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and varying concentrations of Met that may reach hundreds of micromoles/L. *S*-adenosylhomocysteine hydrolase (SAHH) deficiency may manifest by fetal hydrops. Typical features include myopathy (elevated creatine kinase and hypotonia that may lead to respiratory failure), moderate to severe liver disease (elevated aminotransferases, coagulopathy, and even hepatocellular carcinoma), and neuropsychiatric complications (developmental delay, behavioral problems, myelination delay, and hypoplasia of cerebellum, pons or corpus callosum). Efficacy of dietary and pharmacological treatment (Met restriction, phosphatidylcholine and *N*-acetylcysteine administration) is unclear, liver transplant may be beneficial (11).

Disorders of Hcy Remethylation

General characteristics

The remethylation pathway uses, in a coordinated fashion, both betaine: homocysteine methyltransferase (BHMT) and methionine synthase (MTR) to convert Hcy back to Met using methyl group donors betaine and N^5-methyltetrahydrofolate (originating from 1-carbon metabolism), respectively. Met synthase is expressed in many tissues and requires methionine synthase reductase (MTRR) as a redox maintenance system. Nutritional folate and vitamin B-12 deficiencies (48, 49), as well as genetic defects, may impair the remethylation pathway with its complex machinery of folate and cobalamin transporters, and catalytic enzymes requiring vitamin-derived cofactors or coreactants. Remethylation defects lead to the accumulation of Hcy, decreased production of Met and AdoMet, and disturbed 1-carbon homeostasis manifesting predominantly in rapidly growing cells. These biochemical alterations have been implicated in clinical complications such as thromboembolism, neuropsychiatric abnormalities, and impaired hematopoiesis, respectively.

Met synthase and Met synthase reductase deficiency

These 2 biochemically and clinically similar disorders (50) show markedly elevated Hcy in plasma and urine and normal to decreased Met, AdoMet may be normal to low in blood and cerebrospinal fluid, and cystathionine may be increased. Patients suffer from neuropsychiatric and ocular complications (developmental delay, white matter abnormalities with spasticity, epilepsy, nystagmus, and retinopathy), thromboembolic events, failure to thrive, and hematological disturbances (macrocytic/megaloblastic anemia and neutropenia with hypersegmented neutrophils). Therapy includes the administration of betaine, and possibly of AdoMet and large doses of hydroxocobalamin that may partially rescue the enzyme activity in some patients (50, 51).

Genetic disorders of folate and cobalamin transport and metabolism

This large heterogenous group of disorders is caused by genetic defects in intestinal absorption, interorgan transport, intracellular trafficking, and the conversion of folate- or vitamin B_{12}-derived intermediates; readers are referred to other review articles for details (7, 52, 53). Disorders affecting folate availability and N^5-methyltetrahydrofolate synthesis are biochemically and clinically similar to the above-described MTR and MTRR deficiency, with the exception of methylenentetrahydrofolate reductase deficiency that does not exhibit macrocytic/megaloblastic anemia (54). Transport and trafficking defects of vitamin B_{12}, and disorders in the early steps of intracellular cobalamin metabolism impair synthesis of both methylcobalamin and adenosylcobalamin (needed for the conversion of methylmalonyl-CoA into the citric acid cycle intermediate succinate). These diseases manifest not only by the complications described above for MTR/MTRR deficiencies but also by other problems such as failure to thrive and hemolytic uremic syndrome (52, 55, 56). Therapy depends on the underlying genetic defect and may include the administration of folic acid, calcium folinate, or hydroxocobalamin, and of betaine and riboflavin (51, 56).

Disorders in the Transsulfuration Pathway

General characteristics

The proximal part of the human transsulfuration pathway irreversibly converts Hcy to Cys in 2 sequential reactions requiring vitamin B-6-derived pyridoxal 5′-phosphate (PLP) as a cofactor. In addition to their role in transsulfuration, these 2 enzymes also produce H_2S via alternative reactions using 2 molecules of Hcy and/or Cys. Cystathionine β-synthase (CBS) deficiency is often a severe disorder and will be discussed in detail below, cystathionine γ-lyase deficiency has not been consistently associated with adverse clinical outcome and readers are referred to a review elsewhere (44).

CBS deficiency

Classical homocystinuria due to CBS deficiency is the most common and best characterized genetic disorder of SAA metabolism (57). More than 600 patients were described in 1985 (58) and analysis of data on 337 new patients from the European Registry for Homocystinurias and Methylation Defects (E-HOD) registry is now underway (data not shown). The worldwide frequency of this disorder has been estimated as 0.82–1.09 per 100,000 births, although specific populations have higher incidence (9, 10, 59). Many newborn screening programs target classical homocystinuria, mostly by determining elevated Met in dried blood spots (60). More than 160 different pathogenic mutations have been described in patients with CBS deficiency (61). The majority of mutations change a single amino acid residue, often resulting in loss-of-function misfolding (62) that can be corrected by the chaperones (63). Some common mutations (e.g. p.Pro49Leu, p.Ala114Val, and p.Ile278Thr) are rescued in vivo by pyridoxine administration. The propensity of mutants to correction by PLP (probably via its chaperoning effect) plays a role in biochemical and clinical presentations (64, 65). Four different forms of CBS deficiency are known. The multisystemic pyridoxine nonresponsive form manifests in infancy and childhood. The pyridoxine responsive form and the pyridoxine extremely responsive form typically manifest in adulthood by thrombophilia (66, 67, 38). Finally, the partially responsive form exhibits an intermediate phenotype between the severe and milder variants. CBS deficiency leads to the accumulation of Hcy, Met, and AdoMet, and decreased production of cystathionine (42). Although CBS was implicated in producing H_2S, studies in CBS-deficient human patients (39) and mice (T Majtan, J Sokolová, T Ditroi, J Krijt, P Nagy, VKožich, unpublished results, 2020) did not reveal decreased H_2S but rather signs of higher H_2S synthesis and turnover. Met concentrations in untreated CBS-deficient patients may reach hundreds to >1000 of μmol/L and these concentrations may be further increased by providing betaine without ensuring dietary

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Met restriction (68). The severe forms of CBS deficiency affect the nervous system (developmental delay and cognitive problems, behavioral changes, epilepsy, and brain edema in extreme hypermethioninemia) and connective tissue (lenticular myopia progressing into lens dislocation, kyphoscoliosis, marfanoid features, and osteoporosis), and all forms of CBS deficiency may manifest by thromboses and emboli in all vascular beds (58, 66, 42, 69–71). Treatment is effective when started early. Pyridoxine responders require vitamin B-6, whereas patients with partial and nonresponsive forms need a Met-restricted diet and betaine (69, 72). Experimental therapy with a recombinant polyethyleneglycolylated enzyme has been successfully tested in murine models (73, 74).

Disorders of Cys Catabolism

General characteristics

Cys is a precursor for GSH, taurine, and H_2S synthesis (6). Patients with disorders of GSH metabolism usually present with a combination of hemolytic anemia and neurological involvement (75). Genetic disorders in the taurine synthetic pathway have not yet been described in humans, however, mice models of cysteine dioxygenase and cysteine sulfinate decarboxylase deficiency exist (76–79). Disorders of mitochondrial H_2S catabolism are characterized by the accumulation of H_2S, sulfite, and thiosulfate. Excess sulfite reacts with Cys and Hcy forming high amounts of *S*-sulfocysteine and *S*-sulfohomocysteine, and thus depleting plasma total Hcy and Cys (80). Abnormal Met concentrations were not observed, clinical sequelae are multisystemic presenting in the nervous system, vasculature, and connective tissue.

Sulfide: quinone oxidoreductase and persulfide dioxygenase deficiency

Sulfide: quinone oxidoreductase (SQOR) deficiency has been reported in 2 families, patients suffered from episodes of coma with lactic acidosis and basal ganglia lesions suggestive of Leigh syndrome, in 2 cases the episodes were fatal (45), detailed biochemical findings on sulfur compounds are not available. Ethylmalonic encephalopathy due to persulfide dioxygenase deficiency, caused by mutations in the *ETHE1* gene, leads to the accumulation of H_2S, sulfite, and thiosulfate, and to impaired oxidation of short-chain acylcarnitines and lactic acidosis (46, 81, 82). Clinical complications include neurological sequelae (developmental delay, severe cognitive defect, and seizures), failure to thrive, and vascular problems related to the vasodilating effect of H_2S (orthostatic acrocyanosis, petechiae, and severe hemorrhagic diarrhea). Therapy may include metronidazole to decrease H_2S production by the intestinal microbiota and *N*-acetylcysteine as a precursor for the potential H_2S scavenger GSH ; based on previous reports, early liver transplantation appears to be more efficient (83–85).

Sulfite oxidase (SUOX) deficiency can be caused by mutations in the *SUOX* gene (47) or secondarily by genetic defects in the synthesis of the molybdenum cofactor (86, 87), patients accumulate sulfite and related compounds. Isolated SUOX deficiency typically affects the brain (severe atrophy with cyst formation, refractory epilepsy, and severe developmental delay/cognitive impairment) and connective tissue (lens dislocation). Therapy with a low-protein diet combined with a Met- and Cys-free amino acid mixture may be partially successful only in late-onset patients.

Mechanisms associated with abnormal Met metabolism

Genetic defects of a single enzyme in Met metabolism may alter fluxes of many other metabolites leading to dysregulation of distant metabolic and signaling pathways. Several disorders discussed above are associated with long-term exposure to markedly elevated Met concentrations (8, 88–90), whereas the remethylation defects illustrate the effect of low Met availability. **Table 3** shows examples of molecular mechanisms implicated in disorders with altered Met homeostasis. Brain edema and coma associated with extremely high Met concentrations in blood (i.e. typically >800 μmol/L) was observed in MATI/III- and CBS-deficient patients, infants with high nutritional Met intake in formula, and an overdose during a Met loading test (8, 68, 91, 92) probably due to a direct toxic effect of Met on sodium/potassium ATPase (Na^+/K^+ ATPase) (8). A previous review of cases with severe hypermethioninemia and brain edema showed that plasma Met concentrations approaching or above 1000 μmol/L are associated with a high risk of developing encephalopathy and proposed that the contribution of the osmotic effect of betaine is probably negligible (93, 94).

Dietary Treatment and Implications for Lower and Upper Safe Limit of SAA Intake

Recommended daily intake and SAA content in food

Estimates of safe dietary intake of Met and Cys vary widely (95–98). In 2007, the FAO recommended a gradual decrease of combined Met + Cys intake from 31 mg kg^{-1} d^{-1} in infants to ≈ 15 mg kg^{-1} d^{-1} (i.e. 10.4 mg kg^{-1} d^{-1} Met + 4.1 mg kg^{-1} d^{-1} Cys) in adults (99). However, other estimates also accounted for the need of Met and Cys for other reactions in addition to maintaining nitrogen balance and were as high as 88 mg kg^{-1} d^{-1} of combined Met + Cys intake in neonates (98). Dietary proteins contain variable amounts of Met, plant protein may have between <0.5 to >2 g Met per 100 g protein, whereas animal proteins typically contain 2–3 g Met per 100 g protein (100).

Met-restricted diet

Pyridoxine nonresponsive CBS-deficient patients are treated by severe natural protein restriction, administration of amino acid mixtures enriched with Cys, and usually by betaine. Preliminary analysis of data from the E-HOD registry shows that patients on such therapy typically received ≈ 50% and ≈ 60% of natural protein and Met safe intake recommended by the FAO, respectively. The degree of prescribed dietary restriction varied considerably among centers. In a subset of patients with complete data, the combined treatment containing varying amounts of amino acid mixtures, median 53% of safe natural protein intake and median 99 mg kg^{-1} d^{-1} of betaine was associated with elevated blood Met concentrations (median 130, range 12–894 μmol/L). Long-term observational studies indicate that even severe Met restriction sustains high Met concentrations due to blocked transsulfuration and enhanced remethylation (in part also due to betaine treatment), and ensures normal development, growth, and a good long-term clinical outcome (69). However, Met overrestriction in CBS-deficient patients is associated with poor growth.

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TABLE 3 Examples of biochemical and molecular mechanisms in conditions with abnormal methionine metabolism

Disorder/condition	Examples of selected molecular mechanisms
MATI/III deficiency	Animal models: decreased AdoMet content in liver of MATI/III-deficient mice leads to lower PC and sphingomyelin synthesis and to decreased secretion of triglycerides and increased secretion apoB and of lipid-poor VLDL (101), decreased liver synthesis of PC/sphingomyelin resulting in reduced availability of these compounds for brain myelination (21); decreased AdoMet and GSH in liver results in overexpression of number of oncogenes and acute phase inflammatory markers (102), activation of caspase 3 and 8 (103), expansion of liver stem cells; non-AdoMet related functions have been proposed for MATI/III (14)
	Human studies showed normal to decreased blood AdoMet and AdoHcy, normal sarcosine and elevated tHcy (35)
GNMT deficiency	Animal models: GNMT-deficient mice exhibit markedly elevated AdoMet in liver, which leads to increased synthesis of PC and depletion of phosphatidylethanolamine with resulting abnormal diglyceride and triglyceride metabolism (104); elevated AdoMet leads to activation of NF-κB, STAT3 and cyclin signaling pathways (27) and decreased activity of enzymes involved in gluconeogenesis and glycogenolysis (26)
ADK deficiency	Human studies: brain magnetic resonance spectroscopy revealed decreased choline content with normal creatine, and MRI showed delayed myelination (28)
	Animal studies: brain-specific ADK deficiency in mice resulted in adenosine accumulation and dysfunctional adenosine A_{2A} receptor-dependent synaptic plasticity which is associated with seizures and impaired hippocampus-dependent learning (105)
SAHH deficiency	Animal studies: SAHH is a tumor suppressor, decreased SAAH activity leads to hepatocellular carcinoma (106), administration of SAHH inhibitor leads to elevated AdoHcy, upregulation of ERK1/2 and increased proliferation of vascular smooth muscle cell proliferation resulting in enhanced atherosclerosis (107)
CBS deficiency	Human studies: platelet activation, abnormal von Willebrand factor secretion and other mechanisms including dysregulation of H_2S metabolism have been implicated in vascular thromboembolic complications in CBS-deficient patients (108–110); analysis of H_2S and related sulfur compounds indicated enhanced H_2S production and catabolism (39, 111); increased Hcy thiolactone results in *N*-homocysteinylation of different proteins with functional consequences (112–114)
	Animal studies: murine model exhibited elevated blood concentrations of von Willebrand factor (115); liver of CBS-deficient mice exhibited dysregulation of remethylation enzymes (116), ATP binding cassette proteins and nuclear hormone receptors (117) and complex changes in profile of phospholipids due to abnormal methylation (118); analysis of brain showed upregulation of serine/threonine kinase DyrkA1 and AdoHcy hydrolase (119); increased Hcy thiolactone results in *N*-homocysteinylation of proteins as reviewed elsewhere
RMD	Human studies: magnetic resonance spectroscopy revealed decreased brain choline content (120); fibroblast and lymphocyte studies showed increased ROS production and endoplasmic reticulum stress, enhanced apoptosis and mitophagy, mislocalization of RNA binding proteins and altered calcium and glutathione homeostasis (121–123)
	Animal studies: murine liver exhibited complex changes in phospholipid profiles (118) and murine brains exhibited decreased AdoMet, choline, and betaine concentrations and dysregulation of synaptic markers associated with hippocampus-related learning problems (120, 124, 125)
Hyperhomocysteinemia	Hcy disrupts collagen crosslinking, increases osteoclast activity and production of advanced glycosylation end products (126), and impairs response of endothelial cells to injury via dysregulated TNF/NF-κB signaling (127)
Increased dietary Met intake	Human studies: increased Met intake in infant formula resulted in some infants with brain edema probably due to inhibition of Na^+/K^+ ATPase (92)
	Animal studies: chronic administration of Met-rich diet in rats elicited toxic effects, since toxicological markers correlated best with Hcy concentrations, Hcy was implicated as possible mediator of toxic effects (90)

ADK, adenosine kinase; AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; CBS, cystathionine β-synthase; ERK, extracellular signal-regulated kinases; GNMT, glycine *N*-methyltransferase; GSH, glutathione; H_2S, hydrogen sulfide; Hcy, homocysteine; MATI/III, methionine adenosyltransferase I/III; Met, methionine; Na^+/K^+ ATPase, sodium/potassium ATPase; PC, phosphatidylcholine; RMD, remethylation defects; ROS, reactive oxygen species; SAHH, *S*-adenosylhomocysteine hydrolase; STAT3, signal transducer and activator of transcription 3.

Normal diet in remethylation defects and cystinuria

The remethylation pathway provides Met and AdoMet as demonstrated by decreased concentrations of these metabolites, and adverse clinical outcome in patients with remethylation defects on normal dietary Met intake. Interestingly, patients with cystinuria due to impaired renal Cys reabsorption do not exhibit laboratory signs of SAA deficiency despite losing $\approx$ 5–15 mg kg^{-1} d^{-1} of the disulfide cystine in their urine (128, 129). This suggests that the body is able to replenish the missing Cys by its formation in the transsulfuration pathway although a recent animal model study revealed decreased concentration of GSH (130).

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References

1. Aledo JC. Methionine in proteins: the Cinderella of the proteinogenic amino acids. Protein Sci 2019;28:1785–96.
2. Benevenga NJ. Consideration of betaine and one-carbon sources of N5-methyltetrahydrofolate for use in homocystinuria and neural tube defects. Am J Clin Nutr 2007;85:946–9.
3. Petrossian TC, Clarke SG. Uncovering the human methyltransferasome. Mol Cell Proteomics 2011;10(1): M110.000976.
4. Pace NJ, Weerapana E. Diverse functional roles of reactive cysteines. ACS Chem Biol 2013;8:283–96.

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5. Shannon DA, Weerapana E. Orphan PTMs: rare, yet functionally important modifications of cysteine. Biopolymers 2014;101:156–64.
6. Stipanuk MH. Metabolism of sulfur-containing amino acids – how the body copes with excess methionine, cysteine and sulfide. J Nutr 2020;150(Suppl 10):2494S–505S.
7. Froese DS, Fowler B, Baumgartner MR. Vitamin B12, folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation. J Inherit Metab Dis 2019;42:673–85.
8. Mudd SH. Hypermethioninemias of genetic and non-genetic origin: a review. Am J Med Genet 2011;157:3–32.
9. Gan-Schreier H, Kebbewar M, Fang-Hoffmann J, Wilrich J, Abdoh G, Ben-Omran T, Shahbek N, Bener A, Al Rifai H, Al Khal AL, et al. Newborn population screening for classic homocystinuria by determination of total homocysteine from Guthrie cards. J Pediatr 2010;156:427–32.
10. Moorthie S, Cameron L, Sagoo GS, Bonham JR, Burton H. Systematic review and meta-analysis to estimate the birth prevalence of five inherited metabolic diseases. J Inherit Metab Dis 2014;37: 889–98.
11. Baric I, Staufner C, Augoustides-Savvopoulou P, Chien YH, Dobbelaere D, Grunert SC, Opladen T, Petkovic Ramadza D, Rakic B, Wedell A, et al. Consensus recommendations for the diagnosis, treatment and follow-up of inherited methylation disorders. J Inherit Metab Dis 2017;40:5–20.
12. Baric I. Inherited disorders in the conversion of methionine to homocysteine. J Inherit Metab Dis 2009;32:459–71.
13. Baric I, Erdol S, Saglam H, Lovric M, Beluzic R, Vugrek O, Blom HJ, Fumic K. Glycine *N*-methyltransferase deficiency: a member of dysmethylating liver disorders? JIMD Rep 2017;31:101–6.
14. Murray B, Peng H, Barbier-Torres L, Robinson AE, Li TWH, Fan W, Tomasi ML, Gottlieb RA, Van Eyk J, Lu Z, et al. Methionine adenosyltransferase alpha1 is targeted to the mitochondrial matrix and interacts with cytochrome P450 2E1 to lower its expression. Hepatology 2019;70(6):2018–34.
15. Mudd SH, Brosnan JT, Brosnan ME, Jacobs RL, Stabler SP, Allen RH, Vance DE, Wagner C. Methyl balance and transmethylation fluxes in humans. Am J Clin Nutr 2007;85:19–25.
16. Chamberlin ME, Ubagai T, Mudd SH, Thomas J, Pao VY, Nguyen TK, Levy HL, Greene C, Freehauf C, Chou JY. Methionine adenosyltransferase I/III deficiency: novel mutations and clinical variations. Am J Hum Genet 2000;66:347–55.
17. Chien YH, Abdenur JE, Baronio F, Bannick AA, Corrales F, Couce M, Donner MG, Ficicioglu C, Freehauf C, Frithiof D, et al. Mudd's disease (MAT I/III deficiency): a survey of data for MAT1A homozygotes and compound heterozygotes. Orphanet J Rare Dis 2015;10:99.
18. Chadwick S, Fitzgerald K, Weiss B, Ficicioglu C. Thirteen patients with MAT1A mutations detected through newborn screening: 13 years' experience. JIMD Rep 2014;14:71–6.
19. Couce ML, Boveda MD, Castineiras DE, Corrales FJ, Mora MI, Fraga JM, Mudd SH. Hypermethioninaemia due to methionine adenosyltransferase I/III (MAT I/III) deficiency: diagnosis in an expanded neonatal screening programme. J Inherit Metab Dis 2008;31(Suppl 2):S233–9.
20. Martins E, Marcao A, Bandeira A, Fonseca H, Nogueira C, Vilarinho L. Methionine adenosyltransferase I/III deficiency in Portugal: high frequency of a dominantly inherited form in a small area of Douro high lands. JIMD Rep 2012;6:107–12.
21. Chamberlin ME, Ubagai T, Mudd SH, Wilson WG, Leonard JV, Chou JY. Demyelination of the brain is associated with methionine adenosyltransferase I/III deficiency. J Clin Invest 1996;98:1021–7.
22. Guo DC, Gong L, Regalado ES, Santos-Cortez RL, Zhao R, Cai B, Veeraraghavan S, Prakash SK, Johnson RJ, Muilenburg A, et al. MAT2A mutations predispose individuals to thoracic aortic aneurysms. Am J Hum Genet 2015;96:170–7.
23. Pol A, Renkema GH, Tangerman A, Winkel EG, Engelke UF, de Brouwer APM, Lloyd KC, Araiza RS, van den Heuvel L, Omran H, et al. Mutations in SELENBP1, encoding a novel human methanethiol oxidase, cause extraoral halitosis. Nat Genet 2018;50:120–9.
24. Luka Z, Mudd SH, Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. J Biol Chem 2009;284:22507–11.
25. Augoustides-Savvopoulou P, Luka Z, Karyda S, Stabler SP, Allen RH, Patsiaoura K, Wagner C, Mudd SH. Glycine N-methyltransferase deficiency: a new patient with a novel mutation. J Inherit Metab Dis 2003;26:745–59.
26. Liu SP, Li YS, Chen YJ, Chiang EP, Li AF, Lee YH, Tsai TF, Hsiao M, Huang SF, Chen YM. Glycine N-methyltransferase-/- mice develop chronic hepatitis and glycogen storage disease in the liver. Hepatology 2007;46:1413–25.
27. Varela-Rey M, Fernandez-Ramos D, Martinez-Lopez N, Embade N, Gomez-Santos L, Beraza N, Vazquez-Chantada M, Rodriguez J, Luka Z, Wagner C, et al. Impaired liver regeneration in mice lacking glycine N-methyltransferase. Hepatology 2009;50:443–52.
28. Staufner C, Blom HJ, Dionisi-Vici C, Freisinger P, Makhseed N, Ballhausen D, Kolker S, Hoffmann GF, Harting I. MRI and (1)H-MRS in adenosine kinase deficiency. Neuroradiology 2016;58:697–703.
29. Staufner C, Lindner M, Dionisi-Vici C, Freisinger P, Dobbelaere D, Douillard C, Makhseed N, Straub BK, Kahrizi K, Ballhausen D, et al. Adenosine kinase deficiency: expanding the clinical spectrum and evaluating therapeutic options. J Inherit Metab Dis 2016;39:273–83.
30. Baric I, Fumic K, Glenn B, Cuk M, Schulze A, Finkelstein JD, James SJ, Mejaski-Bosnjak V, Pazanin L, Pogribny IP, et al. S-adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. Proc Natl Acad Sci U S A 2004;101:4234–9.
31. Buist NR, Glenn B, Vugrek O, Wagner C, Stabler S, Allen RH, Pogribny I, Schulze A, Zeisel SH, Baric I, et al. S-adenosylhomocysteine hydrolase deficiency in a 26-year-old man. J Inherit Metab Dis 2006;29:538–45.
32. Honzik T, Magner M, Krijt J, Sokolova J, Vugrek O, Beluzic R, Baric I, Hansikova H, Elleder M, Vesela K, et al. Clinical picture of S-adenosylhomocysteine hydrolase deficiency resembles phosphomannomutase 2 deficiency. Mol Genet Metab 2012;107:611–3.
33. Stender S, Chakrabarti RS, Xing C, Gotway G, Cohen JC, Hobbs HH. Adult-onset liver disease and hepatocellular carcinoma in S-adenosylhomocysteine hydrolase deficiency. Mol Genet Metab 2015;116:269–74.
34. Inborn errors of metabolism knowledgebase. Available from: http://iembase.org/.
35. Stabler SP, Steegborn C, Wahl MC, Oliveriusova J, Kraus JP, Allen RH, Wagner C, Mudd SH. Elevated plasma total homocysteine in severe methionine adenosyltransferase I/III deficiency. Metabolism 2002;51:981–8.
36. Kožich V, Morris AAM, Blom HJ. Disorders of sulfur amino acid metabolism. In: Saudubray J-M, Baumgartner MR, Walter J , editors. Inborn metabolic diseases: diagnosis and treatment. Berlin, Heidelberg: Springer Berlin Heidelberg; 2016. pp. 309–20.
37. Barić I, Kožich V, Fowler B. Disorders of sulfur amino acid and hydrogen sulfide metabolism. In: Blau N, Dionisi Vici C, Ferreira C, Vianey-Saban C, van Karnebeek K , editors. Physician's guide to the laboratory diagnosis, treatment, and follow-up of inherited metabolic diseases. 2nd ed. Berlin, Heidelberg: Springer Berlin Heidelberg; in press.
38. Stabler SP, Korson M, Jethva R, Allen RH, Kraus JP, Spector EB, Wagner C, Mudd SH. Metabolic profiling of total homocysteine and related compounds in hyperhomocysteinemia: utility and limitations in diagnosing the cause of puzzling thrombophilia in a family. JIMD Rep 2013;11:149–63.
39. Kozich V, Ditroi T, Sokolova J, Krizkova M, Krijt J, Jesina P, Nagy P. Metabolism of sulfur compounds in homocystinurias. Br J Pharmacol 2019;176:594–606.
40. Scaglia F, Blau N. Disorders of folate metabolism and transport. In: Blau N, Duran M, Gibson KM, Dionisi Vici C , editors. Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. pp. 167–78.
41. Baumgartner MR, Fowler B. Vitamin B12 disorders. In: Blau N, Duran M, Gibson KM, Dionisi Vici C , editors. Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. pp. 205–18.
42. Orendac M, Zeman J, Stabler SP, Allen RH, Kraus JP, Bodamer O, Stockler-Ipsiroglu S, Kvasnicka J, Kozich V. Homocystinuria due to cystathionine beta-synthase deficiency: novel biochemical findings and treatment efficacy. J Inherit Metab Dis 2003;26:761–73.
43. Kozich V, Krizkova M, Ditroi T, Sokolová J, Křížek T, Krijt J, Kohl J, Jesina P, Zeman J, Dionisi-Vici C, et al. P 196: sulfurome

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analysis in patients with homocystinurias and ultra-rare inborn errors of hydrogen sulfide metabolism. J Inherit Metab Dis 42(S1): 125.
44. Kraus JP, Hasek J, Kozich V, Collard R, Venezia S, Janosikova B, Wang J, Stabler SP, Allen RH, Jakobs C, et al. Cystathionine gamma-lyase: clinical, metabolic, genetic, and structural studies. Mol Genet Metab 2009;97:250–9.
45. Friederich MW, Elias AF, Kuster A, Laugwitz L, Larson AA, Landry AP, Ellwood-Digel L, Mirsky DM, Dimmock D, Haven J, et al. Pathogenic variants in SQOR encoding sulfide:quinone oxidoreductase are a potentially treatable cause of Leigh disease. J Inherit Metab Dis 2020, doi 101002/jimd12232.
46. Tiranti V, Viscomi C, Hildebrandt T, Di Meo I, Mineri R, Tiveron C, Levitt MD, Prelle A, Fagiolari G, Rimoldi M, et al. Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. Nat Med 2009;15:200–5.
47. Claerhout H, Witters P, Regal L, Jansen K, Van Hoestenberghe MR, Breckpot J, Vermeersch P. Isolated sulfite oxidase deficiency. J Inherit Metab Dis 2018;41:101–8.
48. Stabler SP. Vitamin B12 deficiency. N Engl J Med 2013;368:2041–2.
49. Green R, Allen LH, Bjorke-Monsen AL, Brito A, Gueant JL, Miller JW, Molloy AM, Nexo E, Stabler S, Toh BH, et al. Vitamin B12 deficiency. Nat Rev Dis Primers 2017;3:17040.
50. Huemer M, Buerer C, Jesina P, Kozich V, Landolt MA, Suormala T, Fowler B, Augoustides-Savvopoulou P, Blair E, Brennerova K, et al. Clinical onset and course, response to treatment and outcome in 24 patients with the cblE or cblG remethylation defect complemented by genetic and in vitro enzyme study data. J Inherit Metab Dis 2015;38:957–67.
51. Huemer M, Diodato D, Schwahn B, Schiff M, Bandeira A, Benoist JF, Burlina A, Cerone R, Couce ML, Garcia-Cazorla A, et al. Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency. J Inherit Metab Dis 2017;40:21–48.
52. Watkins D, Rosenblatt DS. Inborn errors of cobalamin absorption and metabolism. Am J Med Genet 2011;157:33–44.
53. Whitehead VM. Acquired and inherited disorders of cobalamin and folate in children. Br J Haematol 2006;134:125–36.
54. Huemer M, Mulder-Bleile R, Burda P, Froese DS, Suormala T, Zeev BB, Chinnery PF, Dionisi-Vici C, Dobbelaere D, Gokcay G, et al. Clinical pattern, mutations and in vitro residual activity in 33 patients with severe 5, 10 methylenetetrahydrofolate reductase (MTHFR) deficiency. J Inherit Metab Dis 2016;39:115–24.
55. Fischer S, Huemer M, Baumgartner M, Deodato F, Ballhausen D, Boneh A, Burlina AB, Cerone R, Garcia P, Gokcay G, et al. Clinical presentation and outcome in a series of 88 patients with the cblC defect. J Inherit Metab Dis 2014;37:831–40.
56. Huemer M, Diodato D, Martinelli D, Olivieri G, Blom H, Gleich F, Kolker S, Kozich V, Morris AA, Seifert B, et al. Phenotype, treatment practice and outcome in the cobalamin-dependent remethylation disorders and MTHFR deficiency: data from the E-HOD registry. J Inherit Metab Dis 2019;42:333–52.
57. Mudd SH, Finkelstein JD, Irreverre F, Laster L. Homocystinuria: an enzymatic defect. Science 1964;143:1443–5.
58. Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, Andria G, Boers GH, Bromberg IL, Cerone R, et al. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet 1985;37:1–31.
59. Naughten ER, Yap S, Mayne PD. Newborn screening for homocystinuria: Irish and world experience. Eur J Pediatr 1998;157:S84–7.
60. Keller R, Chrastina P, Pavlikova M, Gouveia S, Ribes A, Kolker S, Blom HJ, Baumgartner MR, Bartl J, Dionisi-Vici C, et al. Newborn screening for homocystinurias: recent recommendations versus current practice. J Inherit Metab Dis 2019;42:128–39.
61. Kozich V, Kraus JP, Majtan T. Cystathionine β-synthase (CBS) deficiency: genetics. In: eLS. Chichester: John Wiley and Sons; 2018. doi: 10.1002/9780470015902.a0005935.pub3.
62. Kozich V, Sokolova J, Klatovska V, Krijt J, Janosik M, Jelinek K, Kraus JP. Cystathionine beta-synthase mutations: effect of mutation topology on folding and activity. Hum Mutat 2010;31:809–19.
63. Melenovska P, Kopecka J, Krijt J, Hnizda A, Rakova K, Janosik M, Wilcken B, Kozich V. Chaperone therapy for homocystinuria: the rescue of CBS mutations by heme arginate. J Inherit Metab Dis 2015;38:287–94.
64. Majtan T, Pey AL, Gimenez-Mascarell P, Martinez-Cruz LA, Szabo C, Kozich V, Kraus JP. Potential pharmacological chaperones for cystathionine beta-synthase-deficient homocystinuria. Handb Exp Pharmacol 2018;245:345–83.
65. Sun S, Weile J, Verby M, Wu Y, Wang Y, Cote AG, Fotiadou I, Kitaygorodsky J, Vidal M, Rine J, et al. A proactive genotype-to-patient-phenotype map for cystathionine beta-synthase. Genome Med 2020;12:13.
66. Skovby F, Gaustadnes M, Mudd SH. A revisit to the natural history of homocystinuria due to cystathionine beta-synthase deficiency. Mol Genet Metab 2010;99:1–3.
67. Magner M, Krupkova L, Honzik T, Zeman J, Hyanek J, Kozich V. Vascular presentation of cystathionine beta-synthase deficiency in adulthood. J Inherit Metab Dis 2011;34:33–7.
68. Allen J, Power B, Abedin A, Purcell O, Knerr I, Monavari A. Plasma methionine concentrations and incidence of hypermethioninemic encephalopathy during infancy in a large cohort of 36 patients with classical homocystinuria in the Republic of Ireland. JIMD Rep 2019;47:41–6.
69. Morris AA, Kozich V, Santra S, Andria G, Ben-Omran TI, Chakrapani AB, Crushell E, Henderson MJ, Hochuli M, Huemer M, et al. Guidelines for the diagnosis and management of cystathionine beta-synthase deficiency. J Inherit Metab Dis 2017;40:49–74.
70. Yap S, Boers GH, Wilcken B, Wilcken DE, Brenton DP, Lee PJ, Walter JH, Howard PM, Naughten ER. Vascular outcome in patients with homocystinuria due to cystathionine β-synthase deficiency treated chronically. A multicenter observational study. Arterioscler Thromb Vasc Biol 2001;21:2080–5.
71. Yap S, Naughten ER, Wilcken B, Wilcken DE, Boers GH. Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine beta-synthase deficiency: effects of homocysteine-lowering therapy. Semin Thromb Hemost 2000;26 (3):335–40.
72. Adam S, Almeida MF, Carbasius Weber E, Champion H, Chan H, Daly A, Dixon M, Dokoupil K, Egli D, Evans S, et al. Dietary practices in pyridoxine non-responsive homocystinuria: a European survey. Mol Genet Metab 2013;110:454–9.
73. Bublil EM, Majtan T, Park I, Carrillo RS, Hulkova H, Krijt J, Kozich V, Kraus JP. Enzyme replacement with PEGylated cystathionine beta-synthase ameliorates homocystinuria in murine model. J Clin Invest 2016;126:2372–84.
74. Majtan T, Jones W, Jr, Krijt J, Park I, Kruger WD, Kozich V, Bassnett S, Bublil EM, Kraus JP. Enzyme replacement therapy ameliorates multiple symptoms of murine homocystinuria. Mol Ther 2018;26:834–44.
75. Walker V, Wevers RA, Mayatepek E. Trimethylaminuria, dimethylglycine dehydrogenase deficiency and disorders in the metabolism of glutathione. In: Saudubray JM, Baumgartner MR, Walter J , editors. Inborn metabolic disease. Heidelberg: Springer; 2016. pp. 429–37.
76. Stipanuk MH, Jurkowska H, Roman HB, Niewiadomski J, Hirschberger LL. Insights into taurine synthesis and function based on studies with cysteine dioxygenase (CDO1) knockout mice. Adv Exp Med Biol 2015;803:29–39.
77. Hirschberger LL, Daval S, Stover PJ, Stipanuk MH. Murine cysteine dioxygenase gene: structural organization, tissue-specific expression and promoter identification. Gene 2001;277:153–61.
78. Park E, Park SY, Cho IS, Kim BS, Schuller-Levis G. A novel cysteine sulfinic acid decarboxylase knock-out mouse: taurine distribution in various tissues with and without taurine supplementation. Adv Exp Med Biol 2017;975 (Pt 1):461–74.
79. Park E, Park SY, Dobkin C, Schuller-Levis G. Development of a novel cysteine sulfinic acid decarboxylase knockout mouse: dietary taurine reduces neonatal mortality. J Amino Acids 2014;2014:346809.
80. Kohl JB, Mellis AT, Schwarz G. Homeostatic impact of sulfite and hydrogen sulfide on cysteine catabolism. Br J Pharmacol 2019;176:554–70.
81. Di Meo I, Lamperti C, Tiranti V. Ethylmalonic encephalopathy. 2017 Sep 21 In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A , editors. GeneReviews®. Seattle (WA); 1993–2020.

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82. Tiranti V, D'Adamo P, Briem E, Ferrari G, Mineri R, Lamantea E, Mandel H, Balestri P, Garcia-Silva MT, Vollmer B, et al. Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. Am J Hum Genet 2004;74:239–52.
83. Kilic M, Dedeoglu O, Gocmen R, Kesici S, Yuksel D. Successful treatment of a patient with ethylmalonic encephalopathy by intravenous N-acetylcysteine. Metab Brain Dis 2017;32:293–6.
84. Dionisi-Vici C, Diodato D, Torre G, Picca S, Pariante R, Giuseppe Picardo S, Di Meo I, Rizzo C, Tiranti V, Zeviani M, et al. Liver transplant in ethylmalonic encephalopathy: a new treatment for an otherwise fatal disease. Brain 2016;139:1045–51.
85. Tam A, AlDhaheri NS, Mysore K, Tessier ME, Goss J, Fernandez LA, D'Alessandro AM, Schwoerer JS, Rice GM, Elsea SH, et al. Improved clinical outcome following liver transplant in patients with ethylmalonic encephalopathy. Am J Med Genet 2019;179:1015–9.
86. Atwal PS, Scaglia F. Molybdenum cofactor deficiency. Mol Genet Metab 2016;117:1–4.
87. Schwahn BC, Van Spronsen FJ, Belaidi AA, Bowhay S, Christodoulou J, Derks TG, Hennermann JB, Jameson E, Konig K, McGregor TL, et al. Efficacy and safety of cyclic pyranopterin monophosphate substitution in severe molybdenum cofactor deficiency type A: a prospective cohort study. Lancet 2015;386:1955–63.
88. Garlick PJ. Toxicity of methionine in humans. J Nutr 2006;136:1722S–5S.
89. Schweinberger BM, Wyse AT. Mechanistic basis of hypermethioninemia. Amino Acids 2016;48:2479–89.
90. Toue S, Kodama R, Amao M, Kawamata Y, Kimura T, Sakai R. Screening of toxicity biomarkers for methionine excess in rats. J Nutr 2006;136:1716S–21S.
91. Cottington EM, LaMantia C, Stabler SP, Allen RH, Tangerman A, Wagner C, Zeisel SH, Mudd SH. Adverse event associated with methionine loading test: a case report. Arterioscler Thromb Vasc Biol 2002;22:1046–50.
92. Harvey Mudd S, Braverman N, Pomper M, Tezcan K, Kronick J, Jayakar P, Garganta C, Ampola MG, Levy HL, McCandless SE, et al. Infantile hypermethioninemia and hyperhomocysteinemia due to high methionine intake: a diagnostic trap. Mol Genet Metab 2003;79:6–16.
93. Kempson SA, Zhou Y, Danbolt NC. The betaine/GABA transporter and betaine: roles in brain, kidney, and liver. Front Physiol 2014;5:159.
94. Schwahn BC, Scheffner T, Stepman H, Verloo P, Das AM, Fletcher J, Blom HJ, Benoist JF, Barshop BA, Barea JJ, et al. Cystathionine beta synthase deficiency and brain edema associated with methionine excess under betaine supplementation: four new cases and a review of the evidence. JIMD Rep 2020;52:3–10.
95. Di Buono M, Wykes LJ, Cole DE, Ball RO, Pencharz PB. Regulation of sulfur amino acid metabolism in men in response to changes in sulfur amino acid intakes. J Nutr 2003;133:733–9.
96. Huang L, Hogewind-Schoonenboom JE, van Dongen MJ, de Groof F, Voortman GJ, Schierbeek H, Twisk JW, Vermes A, Chen C, Huang Y, et al. Methionine requirement of the enterally fed term infant in the first month of life in the presence of cysteine. Am J Clin Nutr 2012;95:1048–54.
97. Humayun MA, Turner JM, Elango R, Rafii M, Langos V, Ball RO, Pencharz PB. Minimum methionine requirement and cysteine sparing of methionine in healthy school-age children. Am J Clin Nutr 2006;84:1080–5.
98. Robinson JL, Bertolo RF. The pediatric methionine requirement should incorporate remethylation potential and transmethylation demands. Adv Nutr 2016;7:523–34.
99. World Health Organization, Food and Agricultural Organization of the United Nations, United Nations University. Protein and amino acid requirements in human nutrition. World Health Organ Tech Rep Ser 2007;935:1–265.
100. Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB, van Loon LJC. Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids 2018;50:1685–95.
101. Pascale RM, Feo CF, Calvisi DF, Feo F. Deregulation of methionine metabolism as determinant of progression and prognosis of hepatocellular carcinoma. Transl Gastroenterol Hepatol 2018;3:36.
102. Lu SC, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, Avila MA, Kanel G, Mato JM. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc Natl Acad Sci U S A 2001;98:5560–5.
103. Mari M, Colell A, Morales A, Paneda C, Varela-Nieto I, Garcia-Ruiz C, Fernandez-Checa JC. Acidic sphingomyelinase downregulates the liver-specific methionine adenosyltransferase 1A, contributing to tumor necrosis factor-induced lethal hepatitis. J Clin Invest 2004;113:895–904.
104. Martinez-Una M, Varela-Rey M, Cano A, Fernandez-Ares L, Beraza N, Aurrekoetxea I, Martinez-Arranz I, Garcia-Rodriguez JL, Buque X, Mestre D, et al. Excess S-adenosylmethionine reroutes phosphatidylethanolamine towards phosphatidylcholine and triglyceride synthesis. Hepatology 2013;58:1296–305.
105. Sandau US, Colino-Oliveira M, Jones A, Saleumvong B, Coffman SQ, Liu L, Miranda-Lourenco C, Palminha C, Batalha VL, Xu Y, et al. Adenosine kinase deficiency in the brain results in maladaptive synaptic plasticity. J Neurosci 2016;36:12117–28.
106. Leal JF, Ferrer I, Blanco-Aparicio C, Hernandez-Losa J, Ramon YCS, Carnero A, Lleonart ME. S-adenosylhomocysteine hydrolase downregulation contributes to tumorigenesis. Carcinogenesis 2008;29:2089–95.
107. Luo X, Xiao Y, Song F, Yang Y, Xia M, Ling W. Increased plasma S-adenosyl-homocysteine levels induce the proliferation and migration of VSMCs through an oxidative stress-ERK1/2 pathway in apoE(-/-) mice. Cardiovasc Res 2012;95:241–50.
108. Beard RS, Jr, Bearden SE. Vascular complications of cystathionine beta-synthase deficiency: future directions for homocysteine-to-hydrogen sulfide research. Am J Physiol Heart Circ Physiol 2011;300:H13–26.
109. Davi G, Di Minno G, Coppola A, Andria G, Cerbone AM, Madonna P, Tufano A, Falco A, Marchesani P, Ciabattoni G, et al. Oxidative stress and platelet activation in homozygous homocystinuria. Circulation 2001;104:1124–8.
110. Richard E, Gallego-Villar L, Rivera-Barahona A, Oyarzabal A, Perez B, Rodriguez-Pombo P, Desviat LR. Altered redox homeostasis in branched-chain amino acid disorders, organic acidurias, and homocystinuria. Oxid Med Cell Longev 2018;2018:1246069.
111. Kozich V, Krijt J, Sokolova J, Melenovska P, Jesina P, Vozdek R, Majtan T, Kraus JP. Thioethers as markers of hydrogen sulfide production in homocystinurias. Biochimie 2016;126:14–20.
112. Perla-Kajan J, Utyro O, Rusek M, Malinowska A, Sitkiewicz E, Jakubowski H. N-Homocysteinylation impairs collagen cross-linking in cystathionine beta-synthase-deficient mice: a novel mechanism of connective tissue abnormalities. FASEB J 2016;30:3810–21.
113. Sikora M, Lewandowska I, Kupc M, Kubalska J, Graban A, Marczak L, Kazmierski R, Jakubowski H. Serum proteome alterations in human cystathionine β-synthase deficiency and ischemic stroke subtypes. Int J Mol Sci 2019;20(12):3096.
114. Jakubowski H. Homocysteine modification in protein structure/function and human disease. Physiol Rev 2019;99:555–604.
115. Denis CV, Terraube V, Robert K, Janel N. Elevated plasma von Willebrand factor in a murine model of severe hyperhomocysteinemia. Thromb Haemost 2003;90:362–4.
116. Alberto JM, Hamelet J, Noll C, Blaise S, Bronowicki JP, Gueant JL, Delabar JM, Janel N. Mice deficient in cystathionine beta synthase display altered homocysteine remethylation pathway. Mol Genet Metab 2007;91:396–8.
117. Hamelet J, Demuth K, Paul JL, Delabar JM, Janel N. Hyperhomocysteinemia due to cystathionine beta synthase deficiency induces dysregulation of genes involved in hepatic lipid homeostasis in mice. J Hepatol 2007;46:151–9.
118. Ikeda K, Kubo A, Akahoshi N, Yamada H, Miura N, Hishiki T, Nagahata Y, Matsuura T, Suematsu M, Taguchi R, et al. Triacylglycerol/phospholipid molecular species profiling of fatty livers and regenerated non-fatty livers in cystathionine beta-synthase-deficient mice, an animal model for homocysteinemia/homocystinuria. Anal Bioanal Chem 2011;400:1853–63.
119. Planque C, Dairou J, Noll C, Bui LC, Ripoll C, Guedj F, Delabar JM, Janel N. Mice deficient in cystathionine beta synthase display increased Dyrk1A and SAHH activities in brain. J Mol Neurosci 2013;50:1–6.
120. Debray FG, Boulanger Y, Khiat A, Decarie JC, Orquin J, Roy MS, Lortie A, Ramos F, Verhoeven NM, Struys E, et al. Reduced brain choline in homocystinuria due to remethylation defects. Neurology 2008;71:44–9.

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121. Caterino M, Pastore A, Strozziero MG, Di Giovamberardino G, Imperlini E, Scolamiero E, Ingenito L, Boenzi S, Ceravolo F, Martinelli D, et al. The proteome of cblC defect: in vivo elucidation of altered cellular pathways in humans. J Inherit Metab Dis 2015;38:969–79.
122. Ghemrawi R, Arnold C, Battaglia-Hsu SF, Pourie G, Trinh I, Bassila C, Charif R, Wiedemann A, Flayac J, Robert A, et al. SIRT1 activation rescues the mislocalization of RNA-binding proteins and cognitive defects induced by inherited cobalamin disorders. Metabolism 2019;101:153992.
123. Martinez-Pizarro A, Desviat LR, Ugarte M, Perez B, Richard E. Endoplasmic reticulum stress and autophagy in homocystinuria patients with remethylation defects. PLoS One 2016;11:e0150357.
124. Bahous RH, Cosin-Tomas M, Deng L, Leclerc D, Malysheva O, Ho MK, Pallas M, Kaliman P, Bedell BJ, Caudill MA, et al. Early manifestations of brain aging in mice due to low dietary folate and mild MTHFR deficiency. Mol Neurobiol 2019;56:4175–91.
125. Jadavji NM, Bahous RH, Deng L, Malysheva O, Grand'maison M, Bedell BJ, Caudill MA, Rozen R. Mouse model for deficiency of methionine synthase reductase exhibits short-term memory impairment and disturbances in brain choline metabolism. Biochem J 2014;461:205–12.
126. Saito M, Marumo K. The effects of homocysteine on the skeleton. Curr Osteoporos Rep 2018;16:554–60.
127. Roth J, Goebeler M, Ludwig S, Wagner L, Kilian K, Sorg C, Harms E, Schulze-Osthoff K, Koch H. Homocysteine inhibits tumor necrosis factor-induced activation of endothelium via modulation of nuclear factor-kappa b activity. Biochim Biophys Acta 2001;1540:154–65.
128. Sahota A, Tischfield JA, Goldfarb DS, Ward MD, Hu L. Cystinuria: genetic aspects, mouse models, and a new approach to therapy. Urolithiasis 2019;47:57–66.
129. Malieckal DA, Modersitzki F, Mara K, Enders FT, Asplin JR, Goldfarb DS. Effect of increasing doses of cystine-binding thiol drugs on cystine capacity in patients with cystinuria. Urolithiasis 2019;47:549–55.
130. Woodard LE, Welch RC, Veach RA, Beckermann TM, Sha F, Weinman EJ, Ikizler TA, Tischfield JA, Sahota A, Wilson MH. Metabolic consequences of cystinuria. BMC Nephrol 2019;20:227.

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